pseudouridine was prepared using the ribonuclease-catalyzed reaction described by Heppel *et al.* (1955). Cytidine 2',3'-cyclic phosphate (0.1 mmole) and pseudouridine (100 mg) were dissolved in 0.015 M Tris-chloride buffer (pH 7; 5 ml) and treated with pancreatic ribonuclease (0.1 mg) at 37° for 75 min. The mixture was then shaken with isoamyl alcohol (0.6 ml) and chloroform (0.15 ml) and streaked on Whatman No. 3MM chromatographic paper (66 cm). The components of the mixture were separated with solvent system B and the dinucleoside phosphate band was cut out and eluted (yield, 30 ODU). This product was found to be completely degradable by ribonuclease to cytidine 3'-phosphate and pseudouridine. The dinucleoside phosphate (20 ODU) was dissolved in 0.05 M sodium borate buffer (pH 8.5; 0.3 ml) and treated with Cmc-*p*-toluenesulfonate (40 mg). The mixture was allowed to stand for 20 hr. At this time paper chromatography in solvent systems A and B showed that about 70% of the material had been converted to Cmc derivatives. The dinucleoside phosphate and its mono-Cmc derivative had R_F values 0.41 and 0.61 (solvent system A); 0.18 and 0.26 (solvent system B), respectively. The mixture was treated with concentrated ammonia for 2 hr and then applied to Whatman No. 3MM chromatographic paper and then separated in solvent system B. The Cmc-dinucleoside phosphate (6 ODU) was dissolved in 0.02 M sodium phosphate buffer (pH 7.0; 0.2 ml) and treated with pancreatic ribonuclease (2.5 μg) at 37° for 4 hr. Paper chromatography in solvent system A showed that about 70% of the Cmc derivative had been converted into a mixture of cytidine 3'-phosphate and Cmc-pseudouridine. With twice the amount of enzyme under the same conditions it was possible to hydrolyze all the dinucleoside phosphate to its components.

Chromatography. Paper chromatography was carried out by the descending technique on Whatman No. 1- or 3MM paper with the solvent systems: (A) ethyl alcohol (70 ml)–1 M ammonium acetate (pH 7; 30 ml) and (B) isopropyl alcohol (70 ml)–concentrated ammonia (10 ml)–water (20 ml).

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A Novel Prostaglandin Derivative Formed from Arachidonic Acid by Rat Stomach Homogenates*

C. Pace-Asciak† and L. S. Wolfe‡

ABSTRACT: A novel derivative of prostanoic acid was isolated during the biosynthetic conversion of arachiodonic acid into prostaglandins by rat stomach homogenates. The structure proposed is 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid (I). The elucidation of this structure was based on infrared and nuclear magnetic resonance spectroscopy, mass spectrometry of several derivatives, and products obtained from oxidative ozonolysis. Evidence for the occurrence in minor amounts of an isomer of I, *i.e.*, 6(9)-oxy-11,15-dihydroxyprosta-5,13-dienoic acid (II) was obtained from mass spectrometry of products of oxidative ozonolysis. Prostaglandins E_2 and $F_{2\alpha}$ were also isolated in smaller amounts and identified by mass spectrometry. Two pathways for the formation of I are proposed.

Prostaglandins are oxygenated derivatives of prostanoic acid, a cyclopentane trans substituted in the 1,2 position by a C-7 carboxylic acid and a C-8 alkane. Their structures were originally determined on material isolated from sheep seminal vesicles and human seminal fluid (Bergström and Sjövall, 1960; Bergström *et al.*, 1963; Samuelsson, 1963). They were shown to be derived in sheep seminal vesicles from certain essential fatty acids (Bergström *et al.*, 1964; Nugteren *et al.*, 1966; Hamberg and Samuelsson, 1967). Certain fatty acids that are not precursors to prostaglandins have been shown to inhibit their biosynthesis (Pace-Asciak and Wolfe, 1968; Nugteren, 1970). Prostaglandins are widespread in occurrence in mammalian tissues and possess diverse physiological activ-

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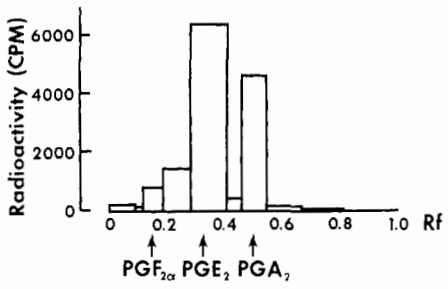

FIGURE 1: Thin-layer radiochromatogram of the ethyl acetate fraction from the silicic acid column purification of the ethanol–water (2:1, v/v) extract (see text for details).

ities (Bergström *et al.*, 1968; Ramwell *et al.*, 1968; Horton, 1969). Recently two new prostaglandin derivatives were isolated from marine invertebrates (Weinheimer and Spraggins, 1969). Several workers have shown that substances with chemical and chromatographic properties of prostaglandins are released into perfusates or superfusates of various tissues, especially after nervous or humoral stimulation (Ramwell *et al.*, 1968; Horton, 1969; Wolfe, 1970). Rat stomach forms and releases prostaglandins during stimulation of the vagus nerve (Coceani *et al.*, 1967; Bennett *et al.*, 1967). Recently Kunze (1970) has shown that 1-^{14}C-labeled PGE_2[1] was formed from 1-^{14}C-labeled arachidonic acid during vascular perfusion of the frog intestine. We have recently shown that homogenates of rat stomach convert arachidonic acid into prostaglandins E_2 and $F_{2\alpha}$ (Pace-Asciak *et al.*, 1968; Pace-Asciak and Wolfe, 1970a,b). We report here the isolation and structure of a new derivative of prostanoic acid (6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid) formed during the transformation of tritium-labeled arachidonic acid into prostaglandins by rat stomach homogenates. A preliminary communication has already appeared (Pace-Asciak and Wolfe, 1970b).

Experimental Section

Incubation Conditions. Adult rats (130 of the Wistar strain, 300–400 g) were used for the following incubations. In the first incubation whole stomachs from 26 rats (starved overnight) were removed, washed with ice-cold buffer (0.05 M KH_2PO_4–NaOH, pH 7.4, containing 0.02 M EDTA), and homogenized in a Servall OmniMixer for 2 min in 200 ml of buffer and tritiated arachidonic acid (125 μg, 8×10^6 dpm/μg, 97+% radiochemical purity). The buffer was preoxygenated prior to the addition of glutathione (56 μg/ml) and recrystallized hydroquinone (0.57 μg/ml). The homogenate was incubated for 20 min at 37° while a gentle stream of oxygen was kept passing through the solution. Four volumes of ethanol containing a few crystals of BHT antioxidant were added to terminate the incubation, and the mixture was stirred for 30 min at room temperature. Filtration through Whatman No. 42 filter paper gave a clear yellow filtrate which was evaporated to dryness. The residue was taken up in chloroform–methanol (2:1, v/v) and thin-layer radiochromatography on silica gel G (chloroform–methanol–acetic acid–water, 90:9:1:0.65, v/v) showed that 9% of the radioactivity migrated in the PGE region. This residue was combined with those obtained from four additional incubations each of 26 rat stomachs to which 25 mg of unlabeled arachidonic acid was added.

Isolation of Prostaglandins and I and II. The combined residues from the five incubations were dissolved in ether, and washed once with 0.03 M HCl and then to neutrality with water. The ether layer was evaporated to dryness and the residue was extracted three times with equal volumes of petroleum ether (bp 30–60°) and ethanol–water (2:1, v/v). The alcohol layers were combined and evaporated to dryness. The residue was transferred to a column of silicic acid (30 g) in chloroform and eluted successively with chloroform, ethyl acetate, and finally methanol. The ethyl acetate fraction contained the least amount of material (120 mg) but the largest amount of radioactivity (7×10^7 dpm, 7% of the total radioactivity used in the experiments). Thin-layer radiochromatography of an aliquot of this fraction (Figure 1) on silica gel G developed as previously showed the majority of radioactivity associated with compounds migrating in the PGE (R_F 0.33) and PGA (R_F 0.50) regions, with a small quantity of counts in the PGF region (R_F 0.15). The total sample was therefore subjected to preparative thin-layer chromatography and the respective zones corresponding to PGA, PGE, and PGF standards were scraped off and eluted with methanol (90%). The residue after evaporation of the methanol was freed from residual silica gel by extraction with ether after acidification. The ether layers were washed to neutrality with water and evaporated to dryness. The material of R_F 0.33 contained a mixture of compounds I, II, and PGE_2. It was therefore treated with 20 ml of ethanol–1 M KOH (1:1, v/v) for 90 min at 23° to convert the PGE_2 into PGB_2 by the procedure of Bergström *et al.* (1963). From the ultraviolet absorption at 280 nm it was calculated that the PGE zone contained 95 μg of PGB_2. (Standards of PGE_2 were used to construct a line relating optical density of PGB_2 to the quantity of PGE_2 used.) The alkali-treated material was diluted with four volumes of water, acidified with 1 M HCl and extracted into ether. The ether layer was washed to neutrality with water and evaporated to dryness. Thin-layer radiochromatography of the residue on silica gel G (system as above) showed 13% of the radioactivity spotted migrating as PGB_2, R_F 0.50, with over 65% of the radioactivity still migrating in the PGE region (R_F 0.33). The two zones were isolated by preparative thin-layer chromatography and the compounds extracted from the silica gel as previously. The material of R_F 0.33 was further purified by argentation thin-layer chromatography (10%) using the AII system of Gréen and Samuelsson (1964) modified to contain double the amount of water (the upper phase of ethyl acetate–methanol–acetic acid–2,2,4-trimethylpentane–water, 110:35:30:10:200, v/v). One major fraction A was obtained R_F 0.50 (approximately 1 mg), composed of two inseparable isomers I and II.

Preparations of Derivatives. Methyl esters of I and II and other isolated prostaglandins were prepared by dissolving the free acids in methanol (0.1 ml) and addition of freshly distilled ethereal diazomethane (0.9 ml). After 60 min in the dark at 23°, the solvent was blown off with a stream of nitrogen and the methyl esters were dissolved in a suitable solvent for further analysis. Trimethylsilyl ethers were prepared by dissolving suitable quantities of methyl esters (usually 20 μg) in 20 μl of Tri-Sil-Z (Pierce Chemical Co., Illinois) in septum covered vials and heated for 5 min in a water bath at 60°. Trimethylsilyl esters were similarly prepared from the free acids. Acetate derivatives were prepared by dissolving the compounds in

[1] Abbreviations used are: PGE_2 and prostaglandin E_2, 9-keto-11α,15-(*S*)-dihydroxyprosta-5,13-dienoic acid; $PGF_{2\alpha}$ and prostaglandin $F_{2\alpha}$, 9α,11α,15(*S*)-trihydroxyprosta-5,13-dienoic acid; PGB_2 and prostaglandin B_2, 9-keto-15(*S*)-hydroxyprosta-5,8(12),13-trienoic acid; BHT, butylated hydroxytoluene.

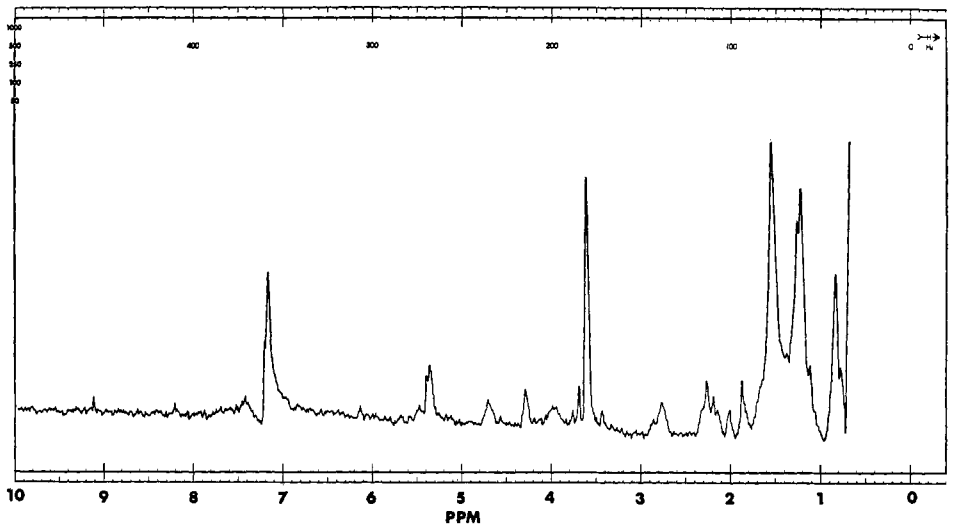

FIGURE 2: 100-MHz nuclear magnetic resonance spectrum of the methyl ester derivative of fraction A (approximately 1 mg) in $CDCl_3$. The spectrum shown is the result of addition of 134 scans by a CAT attachment.

distilled pyridine (0.5 ml), then adding acetic anhydride (0.5 ml). After 16 hr at 23° the mixture was evaporated to dryness *in vacuo*, and the resulting acetate derivatives were dissolved in benzene for subsequent analysis. Hydrogenations were carried out in ethanol (5 ml) and platinum oxide (10 mg) in a microhydrogenator. Sodium borohydride reduction was carried out by dissolving the compound in methanol (5 ml) in an ice bath and gradually adding sodium borohydride (10 mg) to the stirred solution. After the addition was complete, the solution was left stirring at room temperature for 30 min. Water (four volumes) was added, the solution was made weakly acidic with 1 N HCl and extracted with ether.

Oxidative Ozonolysis. The method of Hamberg and Samuelsson (1966) was used on the methyl ester and diacetate derivative of fraction A in chloroform at −25° for 5 min. The resulting blue solution was evaporated to dryness *in vacuo*, acetic acid (1 ml) and 30% hydrogen peroxide (0.5 ml) were added, and the mixture maintained at 50° for 16 hr. The solution was reduced to a volume of approximately 0.5 ml *in vacuo*, then blown almost to dryness with a stream of nitrogen. The residue was converted to the methyl ester derivative, dissolved in 50 μl of chloroform, and analyzed by gas chromatography and mass spectrometry. The temperature of the column (3% SE-30) was programmed from 100° at a rate of 5°/min.

Analytical Methods. Infrared spectra were taken in chloroform in 1-mm cells with a Perkin-Elmer Infracord. Nuclear magnetic resonance spectra were recorded in deuterated chloroform with tetramethylsilane as internal standard on a Varian HA-100 spectrometer using a CAT attachment. Ultraviolet spectra were recorded on a Bausch & Lomb Spectronic 505 recording spectrophotometer. Radioactivity was measured with a Packard 3003 series spectrophotometer using a standard scintillation mixture of toluene–2,5-diphenyloxazole–1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene. Gas chromatography was carried out on an F & M gas chromatograph Model 402B using a 4-ft column of 3% SE-30 ultraphase on Chromosorb W (HP) maintained at 220°. Mass spectra were recorded at 70 eV, unless indicated otherwise, on an LKB 9000 combined gas chromatograph mass spectrometer equipped with rhe same column support as the gas chromatographic analyses and maintained at 260°. Mass spectra were converted into telative intensity diagrams by a PDP-12 computer. Thin-layer plates were scanned for radioactivity with a Packard Model 7201 radiochromatogram scanner using Eastman Chromagram silica gel G sheets.

Materials. All solvents were of the highest commercially available quality and distilled as follows. Diethyl ether was distilled immediately before use, benzene from calcium hydride, ethyl acetate and chloroform from calcium chloride, methanol and ethanol from magnesium turnings and iodine, and pyridine from sodium hydroxide pellets. The following materials were purchased from the suppliers indicated: EDTA (Calbiochem) as the tetrasodium salt, BHT (Mann), reduced glutathione (Sigma), silicic acid HA (Calbiochem), silica gel G (Merck), prostaglandin standards (gift of Dr. J. E. Pike, The Upjohn Co., Kalamazoo), 5,6,8,9,11,12,14,15-[$^3H_8$]arachidonic acid (New England Nuclear), and arachidonic acid (Mann). The arachidonic acid was checked by thin-layer chromatography before use and found to be of over 97% radiochemical purity with the remaining 3% migrating nonspecifically throughout the chromatogram. Diazomethane was generated from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich) in 18 N KOH and ether and then distilled in ether.

Results

Structure of I. Although the chromatographic properties of fraction A suggested a prostaglandin E structure, *i.e.*, a dihydroxyketo compound, it did not react with sodium borohydride indicating that a keto group was not present. The infrared spectrum recorded in chloroform was not of good quality due to the small amounts of purified material available. However, sharp carboxyl absorption at 1710 cm^{-1} (COOH) which shifted to 1730 cm^{-1} ($COOCH_3$) for the methyl ester derivative ruled out the presence of any keto groups in the molecule. Other absorptions were observed at 3400 (br, OH) 2920, 2840 (alkyl). Lack of reaction with potassium hydroxide in ethanol further supports the absence of the β-ketol system of the E prostaglandins. The nuclear magnetic resonance spectrum of the methyl ester derivative (Figure 2) in deuterated chloroform showed peaks at 5.35 ppm due to Δ^{13} and Δ^{7} protons ($PGF_{1\alpha}$ δ 5.50 ppm, Ramwell *et al.*, 1968). Other peaks were observed at 4.30 (proton on C-6 HCO; in adenosine triacetate δ 4.43 ppm, Bhacca *et al.*, 1962, spectrum

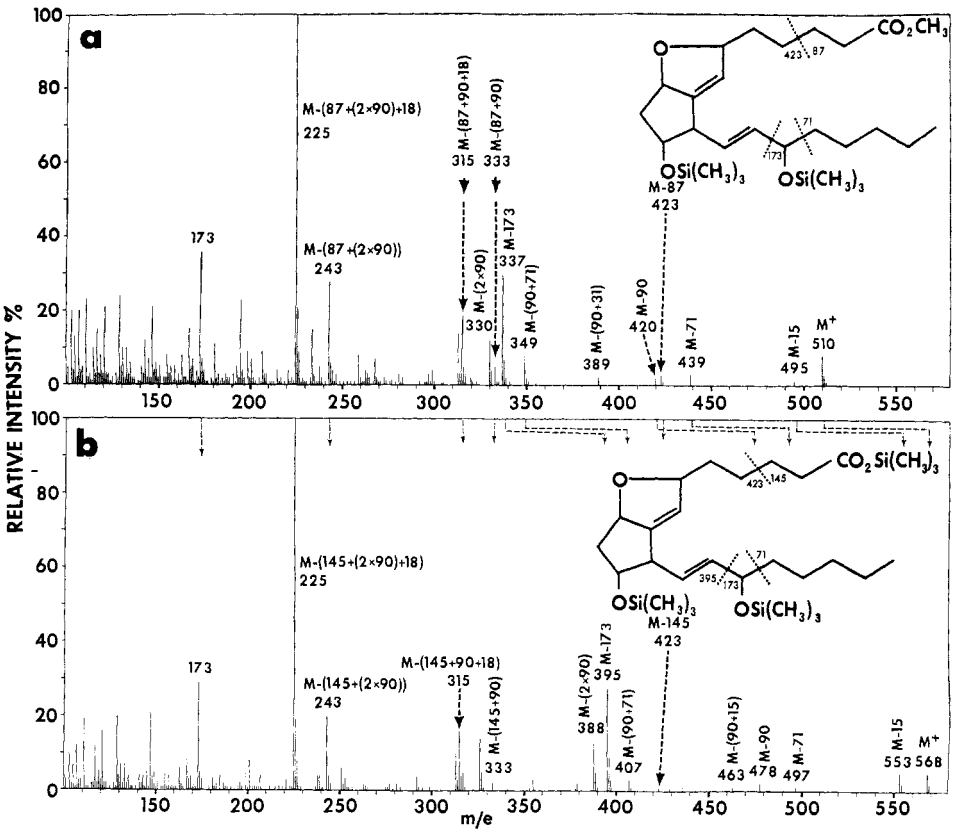

FIGURE 3: Mass spectra of the trimethylsilyl ether derivatives of (a) methyl ester and (b) trimethylsilyl ester of fraction A.

326), 4.00 (*H*COH as in prostaglandins, Ramwell *et al.*, 1968), 3.68 (proton on C-9, HC-O), 3.60 ($COOCH_3$), 2.75 (proton on C-12; in $PGF_{2\beta}$ *ca.* δ 2.70 ppm, Ramwell *et al.*, 1968), and 2.26 ppm (CH_2COO). The peak at 4.65 ppm is interpreted as due to a contaminating Δ^5 isomer identified by oxidative ozonolysis (see below) and is due to the olefinic proton at C-5, OC=CH (in dihydropyran δ 4.65 ppm Bhacca *et al.*, 1962, spectrum 111). The ultraviolet spectrum in ethanol showed only end absorption at 220 nm. In concentrated sulfuric acid, maxima at 315 and 380 nm were observed ($PGF_{2\alpha}$ λ_{max} 318, 400 nm; PGE_2 λ_{max} 260, 315, and 380 nm). In order to obtain structural information on fraction A, it was converted into six derivatives and subjected to gas chromatography–mass spectrometry analysis. The gas chromatographic properties of several derivatives and some of the pertinent mass spectral ions are reported in Table I. The mass spectra of three derivatives appear in Figures 3 and 5. The mass spectrum of the methyl ester derivative showed a molecular ion at *m/e* 366 consistent with the molecular formula $C_{21}H_{34}O_5$. Other ions were observed at 348 (M − 18; loss of H_2O), 335 (M − 31; loss of $\cdot OCH_3$), 330 (M − (2 × 18)), 295 (M − 71; loss of $\cdot C_5H_{11}$), 279 (M − 87; loss of $\cdot(CH_2)_2CO_2CH_3$), 265 (M − 101; loss of $\cdot(CH_2)_3CO_2CH_3$), 251 (M − 115; loss of $\cdot(CH_2)_4CO_2CH_3$), and 222 (M − 144; $CHO(CH_2)_4CO_2CH_3$). The molecular ion shifted to *m/e* 510 in the trimethylsilyl ether and methyl ester derivative indicative of the presence of two hydroxyl groups (Table I and Figure 3a). Other significant ions were at 495 (M − 15; loss of $\cdot CH_3$), 439 (M − 71; loss of $\cdot C_5H_{11}$), 423 (M − 87; loss of $\cdot(CH_2)_2CO_2CH_3$), 420 (M − 90; loss of Me_3SiOH), 389 (M − (90 + 31); loss of Me_3SiOH and $\cdot OCH_3$), 349 (M − (90 + 71); loss of Me_3SiOH and $\cdot C_5H_{11}$), 337 (M − 173; loss of $Me_3SiOCHC_5H_{11}$), 333 (M − (87 + 90)), 330 (M − (2 × 90)), 315 (M − (87 + 90 + 18); loss of $\cdot(CH_2)_2CO_2CH_3$, Me_3SiOH and H_2O), 243 (M − (87 + (2 × 90))), 225 (M − (87 + (2 × 90) + 18)), and 173. In order to obtain more information about the mass spectral fragments containing the carboxyl group in the above spectrum the tri-

TABLE I: Gas–Liquid Chromatographic Properties and Pertinent Mass Spectral Fragments of Six Derivatives of Fraction A.

	Derivative[a] at Retention Times (*C* value)					
	I[b]	II	III	IV	V[b]	VI[b]
Retention						
Fragment[c]	24.20	24.48	24.56	25.40		25.40
M^+	366	510	514	568	450	454
M − (C_3C_1OOR)	279	423		423		
M − (C_5C_1OOR)	251		399			339
M − C_5H_{11}	295	439	443	497		
M − R′OH	348	420	424	478	390	394
M − 2R′OH	330	330	334	388	330	334

[a] (I) Methyl ester, (II) methyl ester and Me_3Si ether, (III) methyl ester Me_3Si ether and hydrogenated, (IV) Me_3Si ether and ester, (V) methyl ester acetate, and (VI) methyl ester acetate and hydrogenated. [b] These mass spectra were recorded on a Perkin-Elmer RMU-6D mass spectrometer at 70 eV by direct inlet sampling (Morgan-Schaffer, Montreal). [c] R refers to the ester derivative and R′ to the hydroxyl derivative used.

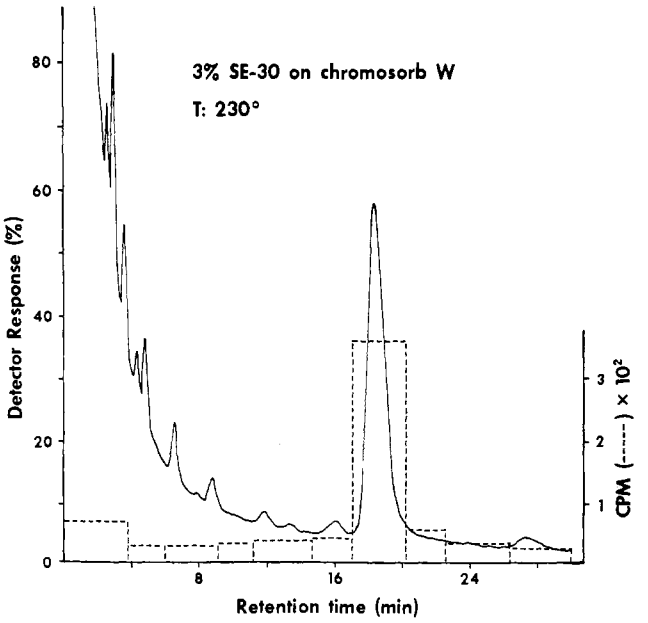

FIGURE 4: Radiogas chromatogram of the trimethylsilyl ether and ester derivative of fraction A.

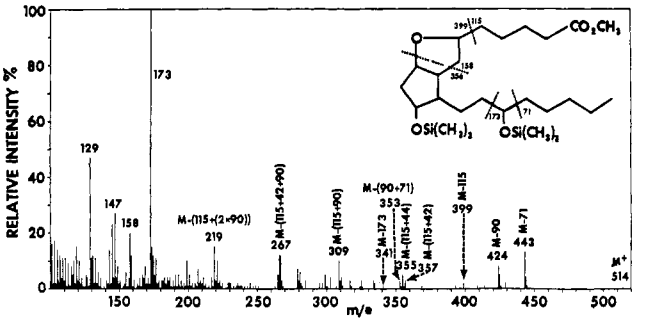

FIGURE 5: Mass spectrum of the trimethylsilyl ether and methyl ester derivative of fraction A after hydrogenation.

methylsilyl ether and ester derivative was prepared (see Figure 4) and its mass spectrum (Table I and Figure 3b) was recorded. The following ions appeared 58 mass units higher than in the corresponding methyl ester derivative indicating that these fragments still contained the ester group: 568 (M+), 553 (M − 15; loss of $\cdot OCH_3$), 497 (M − 71; loss of $\cdot C_5H_{11}$), 478 (M − 90; loss of Me_3SiOH), 463 (M − (90 + 15)), 407 (M − (90 + 71)), 395 (M − 173; loss of $Me_3SiOCHC_5H_{11}$), and 388 (M − (2 × 90)). Fragments involving the loss of the ester group still appeared at *m/e* 423, 333, 315, 243, 225, and 173. Hydrogenation of the methyl ester derivative of fraction A with platinum oxide in ethanol gave a compound which had a retention time on gas chromatography equivalent to a *C* value of 24.56 as the trimethylsilyl ether derivative. Its mass spectrum (Figure 5) showed a molecular ion of low intensity at *m/e* 514, *i.e.*, four mass units higher than the corresponding unsaturated compound indicative of two double bonds in fraction A. Confirmation of the assignment of the molecular ion was obtained from other principal fragments at *m/e* 424 (M − 90; loss of Me_3SiOH), 409 (M − (90 + 15); loss of Me_3SiOH and $\cdot CH_3$), 393 (M − (90 + 31); loss of Me_3SiOH and $\cdot OCH_3$), and 334 (M − (2 + 90)). Fragments of structural significance were observed at 443 (M − 71; loss of $\cdot C_5H_{11}$), 399 (M − 115; loss of $\cdot(CH_2)_4CO_2CH_3$), 357 (M − (115 + 42); loss of $\cdot(CH_2)_4CO_2CH_3$ and CH_2CO), 355 (M − (115 + 44); loss of $\cdot(CH_2)_4CO_2CH_3$ and CH_3CHO), 353 (M − (90 + 71)), 341 (M − 173; loss of $Me_3SiOCHC_5H_{11}$), 309 (M − (115 + 90)), 267 (357 − 90), 219 (M − (115 − (2 + 90))), and 173. The ions resulting from the loss of 71 (C_5H_{11}) and loss of 173 ($Me_3SiOC_6H_{12}$) from the molecular ion in the trimethylsilyl derivatives are consistent with the location of a hydroxyl group in fraction A at position 15 as with the prostaglandins. The difference in the intensity of the ion at *m/e* 173 in the hydrogenated derivative (100%) as compared to the unsaturated derivative (36%) suggests that a double bond is located at position 13,14 in fraction A. This is further supported by oxidative ozonolysis discussed below. If it is assumed that introduction of a hydroxyl group at position 15 and the double bond at position 13 follows the same mechanism as in the formation of prostaglandins (Hamberg and Samuelsson, 1967), then two oxygen atoms would be introduced at positions 9 and 11 together with ring closure at positions 8,12. Since there are only two hydroxyl groups in fraction A and one has already been placed by mass spectrometry at position 15, then one of the oxygen atoms at 9 or 11 must also be a hydroxyl group. Evidence has already been given to show that a keto group is not present in fraction A suggesting therefore that the remaining oxygen atom at 9 or 11 is involved in an ether linkage. Ions involving loss of 115 ($(CH_2)_4COOCH_3$), 115 + 90, and 115 + (2 × 90) from the molecular ion of only the hydrogenated derivative suggest that an oxygen atom is also located at position 6 of fraction A. An ether linkage between positions 6 and 9 would satisfy the above requirements, thereby placing the residual oxygen atom at 11 as a hydroxyl group. The mass spectral evidence for the location of the remaining double bond at position 7,8 is as follows: the ion at *m/e* 423 in the trimethylsilyl ether and methyl ester derivative results from the loss of 87 ($(CH_2)_2COOCH_3$) from the molecular ion. This can be envisaged as taking place after opening of the ether ring with introduction of a double bond at 5,6 (see Figure 6). Ions at *m/e* 333 and 243 result from an additional loss of one and two molecules of trimethylsilanol (90), respectively. The presence of a hydroxyl group in these fragments can be seen by a further loss of 18 to give the base peak at *m/e* 225. The ion at *m/e* 423 is also obtained in the mass spectrum of the trimethylsilyl ether and ester derivative through loss of 145 ($(CH_2)_2COOMe_3Si$) from the molecular ion. Additional loss of 90, (2 × 90), and ((2 × 90) + 18) also occur resulting in the base peak at *m/e* 225. The opening of the ether ring as indicated above is more favorable with a double bond at 7,8 rather than at 6,7 or 8,9. By saturation of the double bond, a different fragmentation takes place giving fragments resulting from cleavage of the 5,6 bond (loss of 115) rather than the 3,4 bond (loss of 87). The presence of two double bonds in fraction A was also confirmed by comparison of the mass spectra of the diacetate and methyl ester derivative of fraction A to the corresponding hydrogenated derivative in which a molecular ion 4 mass units higher (*m/e* 454) than the unsaturated product (*m/e* 450) was observed. Other fragments of significance in the hydrogenated product were observed at *m/e* 394 (M − 60; loss of CH_3CO_2H), 339 (M − 115; loss of $\cdot(CH_2)_4CO_2CH_3$), 334 (M − (2 × 60)), 303 (M − ((2 × 60) + 31); loss of two molecules of CH_3CO_2H and $\cdot OCH_3$), 295 (M − (115 + 44); loss of $\cdot(CH_2)_4CO_2CH_3$ and CH_3CHO), 279 (M − (115 + 60)), 219 (M − (115 + (2 × 60))), 191 (M − (115 + (2 × 60) + 28)), and 175 (M − (115 + (2 × 60) + 44)).

Further evidence for the location of double bonds at 7,8

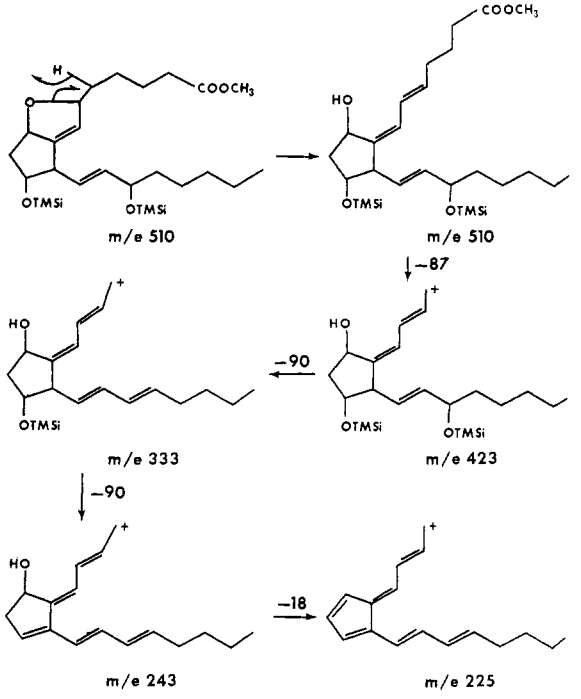

FIGURE 6: Proposed fragmentation pattern of the trimethylsilyl ether and methyl ester of fraction A; structure of ion at *m/e* 225.

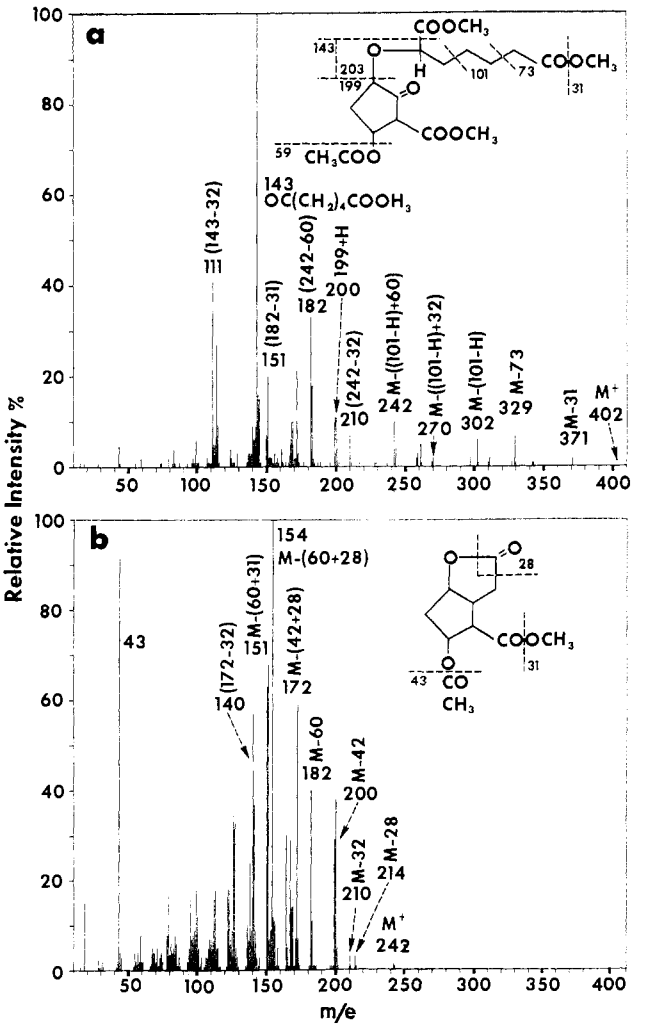

FIGURE 7: Mass spectra of the ring components from the oxidative ozonolysis experiment. (a) Product derived from I and (b) product derived from II. Spectra were recorded at 20 eV.

and 13,14 and a hydroxyl group at 15 was obtained by oxidative ozonolysis of the methyl ester and diacetate derivative of fraction A. Gas chromatography of the oxidation products after methylation gave two major fragments with retention times equivalent to a *C* value of 9.70 and 20.64. The former product was identified as methyl α-acetoxyheptanoate by comparison with the retention time and mass spectrum of an authentic sample prepared by treating the methyl ester and triacetate derivative of prostaglandin $F_{2\alpha}$ in a similar fashion. Mass spectrometry of the second ozonolysis product (*C* value 20.64) showed a weak molecular ion at *m/e* 402 consistent with the remaining portion of the molecule. The mass spectrum was consistent with the structure proposed (Figure 7a). Ions of high intensity appeared at *m/e* 371 (M − 31; loss of $\cdot OCH_3$), 329 (M − 73; loss of $\cdot CH_2CO_2CH_3$), 311 (M − (60 + 31); loss of CH_3CO_2H and $\cdot OCH_3$), 302 (M − (101 − H); loss of $CH_2{=}CHCH_2CO_2CH_3$), 297 (M − (73 + 32); loss of $\cdot CH_2CO_2CH_3$ and CH_3OH), 242 (M − (60 + (101 − H))), 210 (242 − 32), 200 (M − (203 − H); loss of $CO(CO_2CH_3)CH_2CH_2CH_2CH_2CO_2CH_3$), 199 (M − 203), 182 (M − ((2 × 60) + (101 − H)), 143 ($CO(CH_2)_4CO_2CH_3$), and 111 (143 − 32). Two minor oxidation products were also identified as dimethyl glutarate and a product with a *C* value of 14.64. The latter product had a molecular ion at *m/e* 242 with other ions appearing at *m/e* 214 (M − 28; loss of CO), 210 (M − 32; loss of CH_3OH), 200 (M − 42; loss of CH_2CO), 182 (M − 60; loss of CH_3CO_2H), 172 (M − (42 + 28)), 154 (M − (60 + 28)), 151 (M − (60 + 31); loss of CH_3CO_2H and $\cdot OCH_3$), and 140 (M − (42 + 28 + 32)) consistent with the structure proposed (Figure 7b). The formation of these products after oxidative ozonolysis strongly supports the occurrence in fraction A (Figure 8) of the two proposed isomers, 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid (I, the major isomer) and 6(9)-oxy-11,15-dihydroxyprosta-5,13-dienoic acid (II, the minor isomer).

Prostaglandin E_2. The compound of R_F 0.50 produced after alkali treatment of the PGE band exhibited ultraviolet absorption at 278 nm equivalent in intensity to 95 μg of PGB_2. Gas chromatography of the sample after conversion into the methyl ester derivative showed only one peak with a retention time equivalent to 24.2*C*. The mass spectrum showed a fragmentation pattern identical with that obtained with an authentic sample of the methyl ester of PGB_2 with principal fragments at *m/e* 348 (M+), 330 (M − 18; loss of H_2O), 317 (M − 31; loss of $\cdot OCH_3$), 299 (M − (18 + 31)), 287 (M − (18 + 43); loss of H_2O and $\cdot CH_2CH_2CH_3$), 249 (M − (101 − 2H); loss of $CO(CH_2)_4CH_3$), 247 (M − 101; loss of $\cdot CHOH(CH_2)CH_3$), 217 (M − (32 + (101 − 2H)), and 215 (M − 32 + 101)). This indicated that the material of R_F 0.50 was PGB_2 and that PGE_2 was therefore contained in the mixture of R_F 0.33 formed in the biosynthesis experiment.

Prostaglandin $F_{2\alpha}$. The material of R_F 0.15 was converted to the methyl ester and trimethylsilyl ether derivative. Gas chromatography showed a major peak with the same retention time as the methyl ester and trimethylsilyl ether derivative of $PGF_{2\alpha}$ (24.2*C*). The mass spectra of the two compounds were identical, showing ions at *m/e* 584 (M+), 569 (M − 15; loss of $\cdot CH_3$), 513 (M − 71; loss of $\cdot C_5H_{11}$), 494 (M − 90; loss of Me_3SiOH), 479 (M − (90 + 15)), 443 (M − 141; loss of $\cdot CH_2CH{=}CH(CH_2)_3CO_2CH_3$), 423 (M − (90 + 71)), 404

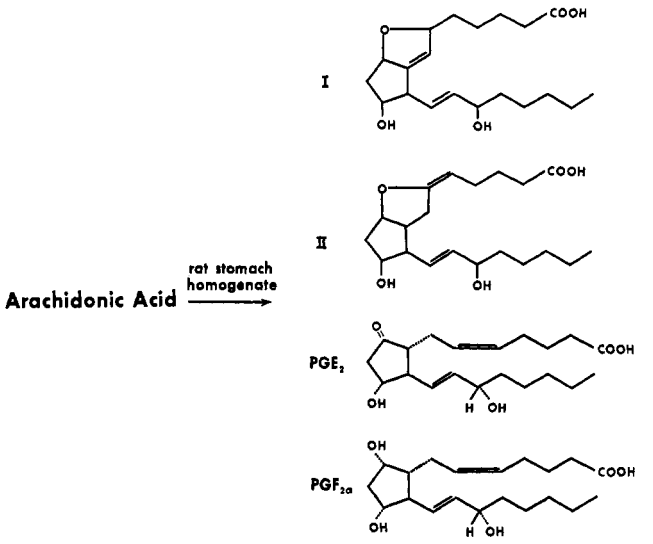

FIGURE 8: Products isolated from the incubation of arachidonic acid with rat stomach homogenates.

(M − (2 × 90)), 397 (M − 187; loss of ·$CH_2CH(OMe_3Si)$-$CH_2(CH_2)_3CH_3$), 353 (M − (90 + 141)), 333 (M − ((2 × 90) + 71)), 307 (M − (90 + 187)), 295 (M − (90 + 199); loss of Me_3SiOH and ·$CH{=}CHCH(OMe_3Si)CH_2(CH_2)_3CH_3$), 217, 191, 173, 147, and 129. The total sample was quantitated by gas chromatography using known quantities of the methyl ester and trimethylsilyl ether derivative of $PGF_{1\alpha}$ and found to contain 70 μg of $PGF_{2\alpha}$.

Discussion

During a study of the biosynthesis of prostaglandins from tritium-labeled arachidonic acid in rat stomach homogenates, a newly labeled compound was isolated in addition to labeled PGE_2 and $PGF_{2\alpha}$ which possessed chromatographic properties similar to the prostaglandins (Pace-Asciak and Wolfe, 1970a,b). We report here its isolation and evidence supporting its structure, 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid (I) (Figure 8). These products were not formed when boiled tissue homogenates were used (C. Pace-Asciak, unpublished data). The formation of PGE_2 and $PGF_{2\alpha}$ demonstrates that the prostaglandin synthetase activity is present in rat stomach. It is not known at this time, however, whether I is also formed and released from intact tissue preparations as has been shown for the prostaglandins (Bennett *et al.*, 1967; Coceani *et al.*, 1967). Since identification of prostaglandins in perfusates of tissues has previously been based on identification by chromatography and quantitation by pharmacological activity, I could very likely have gone undetected due to a much lower biological activity than the normal prostaglandins.[2]

The proposed structure of I is very similar to prostaglandin E_2 and $F_{2\alpha}$, except that the double bond at Δ^5 has been replaced by an oxygen bridge between C-6 and C-9. Two different pathways for the formation of I are possible as shown in Figure 9. One pathway (a) involves homolytic cleavage of the cyclic endoperoxide intermediate (III) of Hamberg and Samuelsson (1967) postulated for the biosynthesis of prostaglandins, followed by attack of the oxygen radical at C-9 on

[2] Tests for pharmacological activity of I were carried out on the gerbil colon by Dr. F. Coceani, The Hospital for Sick Children, Toronto, Canada.

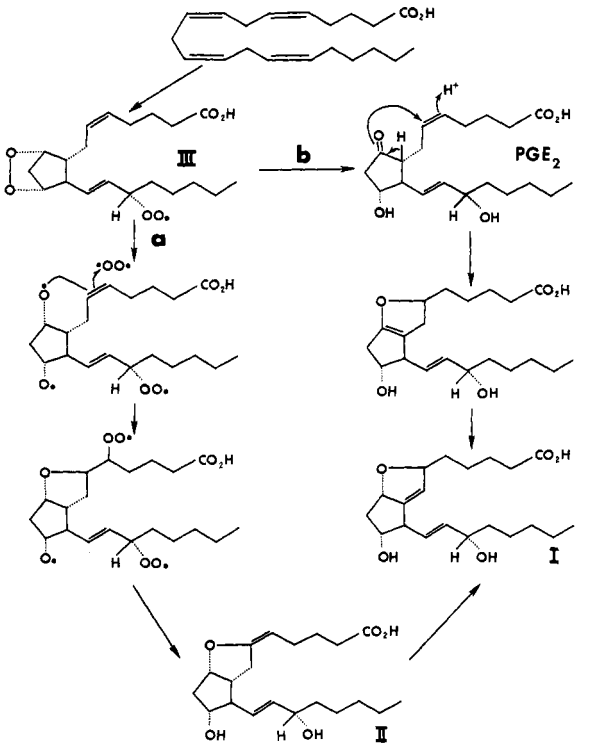

FIGURE 9: Two possible pathways involved in the biosynthesis of I.

the C-6 position of the Δ^5 double bond with hydroperoxidation at C-5. This is followed by elimination of the hydroperoxy group to give II. Isomerization of the double bond to the Δ^7 position would form I. Another possible pathway (b) is from PGE_2, *via* abstraction of the hydrogen atom at C-8, enolization of the keto group at C-9, attack of the resulting enolate ion on the C-6 position of the Δ^5 double bond, and isomerization of the Δ^8 double bond to Δ^7. Further work is being carried out to determine which pathway is involved in the formation of I. Compound II was identified as a minor component by mass spectrometry of products of oxidative ozonolysis. This is suggestive evidence favoring pathway a.

Acknowledgments

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Polyhydroxy Cyclic Ethers Formed from Tritiated Arachidonic Acid by Acetone Powders of Sheep Seminal Vesicles*

C. Pace-Asciak†

ABSTRACT: Three novel derivatives of polyunsaturated fatty acids were isolated during the enzymatic conversion of tritiated arachidonic acid into prostaglandins by acetone powders of sheep seminal vesicles. Two were derived from exogenous tritiated arachidonic acid and are: 9(12)-oxy-8,11,15-trihydroxyeicosa-5,13-dienoic acid (I) and 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid (II). The third compound, 9(12)-oxy-8,11,15-trihydroxyeicosa-13-enoic acid (III), was derived from endogenous eicosatrienoic acid. Compound II was also shown to be formed from arachidonic acid by rat stomach homogenates. The structure of I was elucidated mainly by mass spectrometry of several derivatives and of the products of oxidative ozonolysis. Compounds III and I could be converted into the same compound by catalytic reduction.

The conversion of certain polyunsaturated fatty acids into prostaglandins and their nor and homo derivatives by sheep seminal vesicles has been well documented (Bergström *et al.*, 1964; Wallach, 1965; Kupiecki, 1965; Struijk *et al.*, 1966; Hamberg and Samuelsson, 1967; Beerthuis *et al.*, 1968; Lapidus *et al.*, 1968). During our investigation of the biosynthesis of prostaglandins from tritiated arachidonic by the rat stomach, the major quantity of radioactivity converted into more polar products was found associated with a compound migrating chromatographically like prostaglandin E_2[1] (Pace-Asciak and Wolfe, 1970a). This was shown to be a novel prostanoic acid derivative containing an ether linkage between the C-6 and C-9 positions (Pace-Asciak and Wolfe, 1970b). The present work was carried out to determine whether this compound and/or others were formed in a seminal vesicle preparation known to have a high capacity to form prostaglandins. A preliminary communication has already appeared (Pace-Asciak and Wolfe, 1970c).

Experimental Section

Preparation of Acetone Powder. Seminal vesicles from sheep were obtained from Canada Packers Ltd., Montreal. The glands were dissected at the killing floor, washed with water, and immediately frozen on Dry Ice until used. When 3 kg was collected, acetone powders were prepared in 300 g (fresh weight) lots as follows. The glands were placed in a mortar filled with liquid nitrogen and pulverized by pounding with a pestle. The powdered frozen tissue was poured with liquid nitrogen into precooled acetone (4 l.) and the mixture was stirred at room temperature until a temperature of −20° was reached, then suction filtered through a Büchner funnel. The powder was again extracted with acetone (4 l.) precooled to −20°, and the mixture was stirred at room temperature until a temperature of 4° was reached. The mixture was filtered and finally 400 ml of diethyl ether was passed through the powder

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† Medical Research Council of Canada Scholar.

[1] The abbreviations used are: PGA_1 and prostaglandin A_1, 9-keto-15(*S*)-hydroxyprosta-10,13-dienoic acid; PGA_2 and prostaglandin A_2, 9-keto-15(*S*)-hydroxyprosta-5,10,13-trienoic acid; PGB_2 and prostaglandin B_2, 9-keto-15(*S*)-hydroxyprosta-5,8(12),13-trienoic acid; PGE_1 and prostaglandin E_1, 9-keto-11α,15(*S*)-dihydroxyprost-13-enoic acid; PGE_2 and prostaglandin E_2, 9-keto-11α,15(*S*)-dihydroxyprosta-5,13-dienoic acid; $PGF_{1\alpha}$ and prostaglandin $F_{1\alpha}$, 9α,11α,15(*S*)-trihydroxyprost-5-enoic acid; $PGF_{2\alpha}$ and prostaglandin $F_{2\alpha}$, 9α,11α,15(*S*)-trihydroxyprosta-5,13-dienoic acid; BHT, butylated hydroxytoluene.